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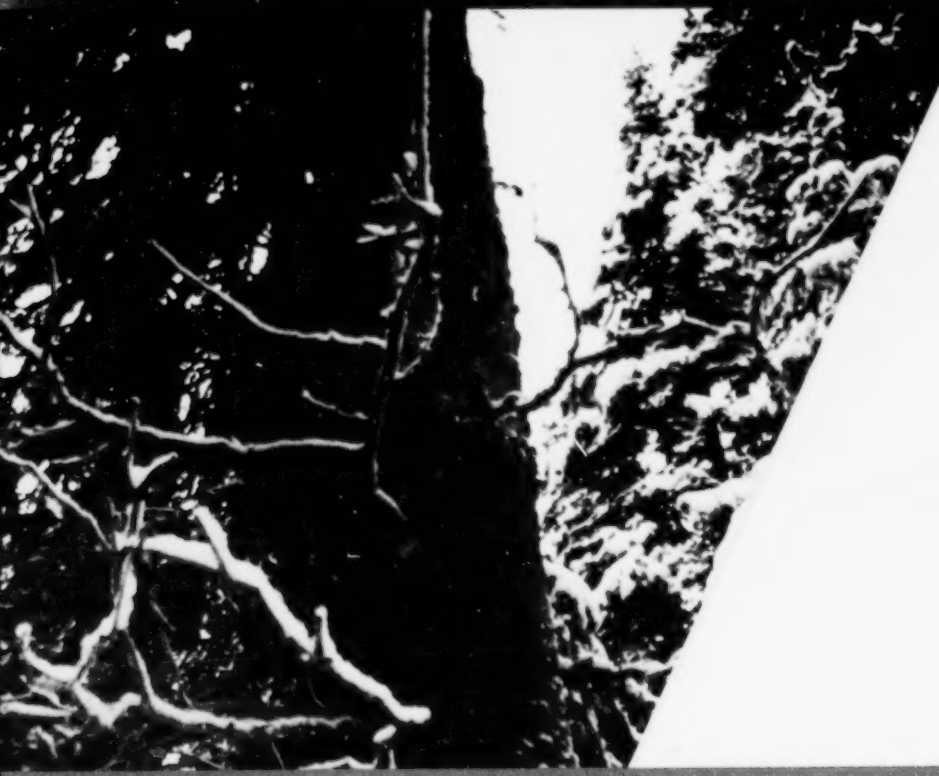

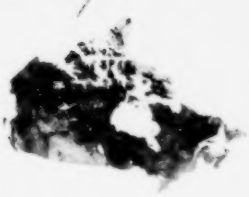

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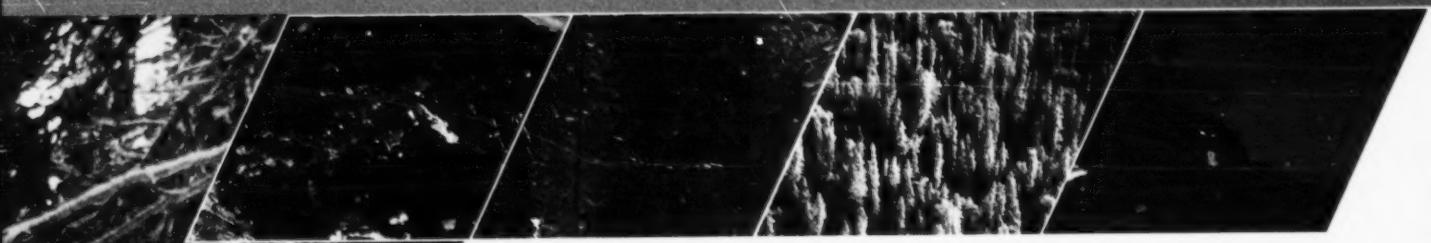

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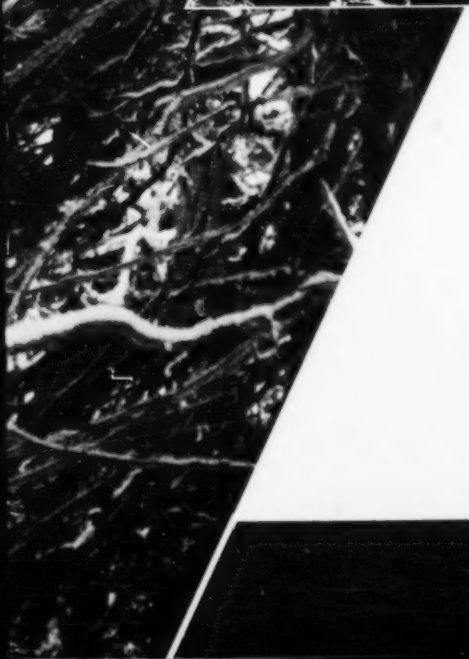

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L. Safranyik, H. S. Whitney, and K. P. Bleiker
Pacific Forestry Centre, Canadian Forest Service
Victoria, British Columbia

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Abstract

Two-year cycle spruce beetle (*Dendroctonus rufipennis* [Kirby]) adults were collected from emergence traps installed on two types of hosts (stumps and windfalls) in two adjacent timber harvesting areas in central British Columbia over a 5-year period to determine the incidence of associated fungi, bacteria, yeasts, nematodes, and mites. There was no difference in either the mean size or the female ratio of spruce beetles between host types or areas. Yeasts and bacteria were the most common associates of the spruce beetle and were isolated from the majority of beetles, regardless of host type. *Pesotum* sp. A, a blue-stain fungus, was the most common filamentous species and was isolated from 63% of the 221 beetles sampled. This fungus appears to be closely associated with the spruce beetle regardless of host type. At least 25 taxa of other filamentous fungi (OFF) were also isolated from beetles. Most of the OFF were common, wind-dispersed species, prevalent in the environment, e.g., *Penicillium* and *Cladosporium* spp., and are likely only incidental associates of the spruce beetle. The OFF were more likely to be isolated from beetles emerging from windfalls than from stumps. This may have been due to higher moisture in windfalls, which promoted the growth of some OFF. The incidence of mites and nematodes associated with the spruce beetle was relatively low; however, only those observed on the exoskeleton were recorded. The association of microorganisms with the spruce beetle did not vary between the two timber harvesting areas sampled in this study. Despite differences in the two types of host material, the spruce beetle is able to maintain a consistent association with yeasts, bacteria, and *Pesotum* sp. A.

Key words: *Dendroctonus rufipennis*, fungi, bacteria, yeasts, nematodes, mites, blue-stain, British Columbia

Résumé

Des dendroctones de l'épinette à cycle bisannuel (*Dendroctonus rufipennis* [Kirby]) adultes ont été collectés sur une période de cinq ans au moyen de pièges d'émergence installés sur deux types d'hôtes (souches et chablis) dans deux zones d'exploitation forestière adjacentes du Centre de la Colombie-Britannique, dans le but de déterminer l'incidence des champignons, bactéries, levures, nématodes et mites associés. Aucune différence n'a été constatée entre les deux types d'hôtes au regard de la taille moyenne ou de la proportion de spécimens femelles du dendroctone de l'épinette. Levures et bactéries constituaient les deux types d'organismes les plus couramment associés au dendroctone de l'épinette et, quel que soit l'hôte, ont été identifiés sur la majorité des spécimens de dendroctones. Pesotum sp. A, un champignon du bleuissement, constituait l'espèce filamenteuse la plus commune et a été identifié sur 63 % des 221 dendroctones échantillonnés. Ce champignon semble être étroitement associé au dendroctone de l'épinette, indépendamment du type d'hôte. Au moins 25 taxa d'autres champignons filamenteux ont également été identifiés sur les dendroctones. La plupart des autres champignons filamenteux, consistant en des espèces communes disséminées par le vent prédominantes dans l'environnement, telles que *Penicillium* et *Cladosporium* spp, ne sont probablement qu'accessoirement associés au dendroctone de l'épinette. Ces autres champignons filamenteux ont été plus souvent identifiés sur les dendroctones des chablis que sur ceux capturés dans les souches. La raison en est peut-être le taux d'humidité plus élevé des chablis qui pourrait avoir favorisé la croissance de certains de ces autres champignons filamenteux. L'incidence des mites et des nématodes associés au dendroctone de l'épinette était relativement faible; toutefois, seuls ont été recensés les spécimens identifiés sur l'exosquelette. L'association des micro-organismes échantillonnés lors de la présente étude du dendroctone de l'épinette était similaire dans les deux zones d'exploitation forestière étudiées. En dépit des différences entre les deux types d'hôtes, le dendroctone de l'épinette parvient à maintenir une association constante avec les levures, les bactéries et *Pesotum* sp. A.

Mots clés: *Dendroctonus rufipennis*, champignons, bactéries, levures, nématodes, mites, bleuissement, la Colombie-Britannique

Contents

Introduction 1

Methods 2

Results 3

Discussion 4

Table 1. The frequency and relative abundance (I'_i) of filamentous fungal taxa and isolated from spruce beetles emerging from stumps (n = 61 beetles) and windfalls (n = 160 beetles). 8

References 9

Appendix A 13

Table A1. Incidence of microorganisms associated with spruce beetles emerging from stumps by sample area, stump, location on stump, and year. 13

Table A2. Incidence of microorganisms associated with spruce beetles emerging from windfalls by sample area, tree, location on windfall, and year 15

Introduction

The spruce beetle, *Dendroctonus rufipennis* (Kirby) (Coleoptera: Curculionidae: Scolytinae), is native to the spruce (*Picea*) forests of North America (Bright 1976). All native spruce can be infested but the major hosts are white spruce (P. *glauca* [Moench] Voss), Engelmann spruce (P. *engelmanni* Parry) and their hybrids. Endemic populations of beetles normally infest weakened or decadent trees, fresh windfalls, logs and logging residue (Dyer and Safranyik 1977). Fresh logging residue such as stumps and cull logs (Dyer and Taylor 1971; Schmid 1977) and wind-felled trees (Wygant and LeJeune 1967) are readily infested by spruce beetles. Under the right conditions for beetle establishment and survival, populations that build up and emerge from these host materials may constitute a significant proportion of the endemic population in a timber harvesting area (Safranyik et al. 1983) and may be precursors to catastrophic outbreaks in apparently healthy trees.

Spruce beetle has a 2-year life cycle throughout much of its range; however, a 1-year life cycle is common in the south, at warm sites, or in exposed logging residue, while a 3-year cycle is possible at colder sites (Schmid and Frye 1977; Safranyik et al. 1983). Local climate and seasonal weather patterns may affect the rate at which the spruce beetle develops, resulting in variation in the duration of its life cycle at a given site. In the 2- and 3-year life cycles, spruce beetle overwinters as larvae in its first and second winters, respectively. All broods overwinter once as teneral adults to become sexually mature (Schmid and Frye 1977). The emergence period is governed by temperature, and typically occurs between May and August with peak emergence in June. It may be advantageous for the spruce beetle to attack trees before the onset of yearly cell division when resin exudation is low and trees are not able to produce new resin in response to invasion by the beetle–microorganism complex (Safranyik et al. 1983).

Mature adult spruce beetles have two colour morphotypes: all black, or two-toned where the head and pronotum are black and the elytra are brown to reddish-brown. Wood (1982) states that older beetles are usually black; however, Linton et al. (1984) indicate that colour may be genetically controlled. The ratio of two-toned to black beetles is approximately 2:1 in large field collections, but it may vary geographically as well as among beetle generations over time (Linton et al. 1984).

Like other bark beetles, spruce beetle is associated with a complex of microorganisms that are introduced into host trees by attacking adults as they construct egg galleries (e.g., Whitney 1982). Potential associates include filamentous fungi, yeasts, bacteria, viruses, nematodes, mites, and protozoans. Ophiostomatoid fungi are particularly well known for their close associations with bark beetles, and they have sticky spores which are adapted for dispersal by insects (Dowding 1969; Paine et al. 1997). This group of filamentous fungi rapidly colonize the phloem and sapwood of successfully attacked trees. Some species are known colloquially as 'blue-staining' because of the dark blue-black colour they impart to the sapwood as their hyphae melanise. Ophiostomatoid fungi may play a role in bark beetle biology by depleting the defences of living trees, conditioning host tree tissues for brood development through moisture or chemical changes, contributing to pheromone synthesis and providing nutritional supplementation (e.g., Whitney 1971; Brand et al. 1976; Safranyik et al. 1983; Lieutier 1993; Nebeker et al. 1993; Solheim et al. 2001; Bleiker and Six 2007). Interactions between bark beetles and microorganisms range from obligate to merely incidental and outcomes of interactions range from commensalism to mutualism through to antagonism (e.g., Whitney 1982; Klepzig et al. 2001). Such interactions, as well as interactions among the various associates themselves, may affect bark beetle fitness and potentially population dynamics (e.g., Bridges 1981; Goldhammer et al. 1990; Coppedge et al. 1995; Ayres et al. 2000; Bleiker and Six 2007, 2009).

In 1972, an 8-year field study of spruce beetle population dynamics was established in central British Columbia in two adjacent timber harvesting areas. As part of this study, each year from 1975 to 1979, we collected newly emerged adult spruce beetles from emergence traps installed on stumps and windfalls. Each year emerged beetles were chosen at random from collections and examined for the presence of filamentous fungi, yeasts, bacteria, mites, and nematodes. Our objective was to provide a survey of the presence and relative abundance of these organisms associated with newly emerged adult spruce beetles.

Methods

The study sites were comprised of two adjacent timber harvesting licenses of 940 ha (A) and 1640 ha (B) on the Naver Forest, about 50 km southeast of Prince George, British Columbia. The stands were comprised of mature (150 yrs) spruce (*Picea glauca x P. engelmanni* hybrid population) and subalpine fir (*Abies lasiocarpa* [Hook] Nutt.) with spruce dominating the overstorey. The average spruce tree diameters (at 1.3 m) greater than 20 cm in areas A and B were 49.5 cm and 52.4 cm, respectively; the corresponding average stand densities were 139.5 and 97.9 stems per ha. Harvesting in both areas was by clearcutting.

Each year during late April and early May, prior to beetle emergence, emergence traps were installed on stumps and windfalls that had been attacked by spruce beetle 2 years previously (i.e., on host material that contained 2-year cycle adult beetles). Each trap consisted of a 4-inch (10.2-cm) diameter clean, cylindrical, metal motor oil can with one open end and a hole cut through the side near the other end to accommodate the perforated screw cap of a 3-dram glass vial. The open ends of the traps were inserted into shallow kerfs cut through the bark and sapwood with a 4-inch-diameter hole saw, so that the glass collection vials hung down. Emergence traps were placed near the top, middle, and base of stumps in the four cardinal directions for a total of 12 traps per stump. On windfalls, traps were placed laterally opposite one another at four equidistant points between the base and the top of the attack for a total of eight traps per windfall. Collections were made at intervals of 2 to 6 days. At each collection, any vials that contained emerged beetles were removed and replaced with a clean empty vial. Clean, new caps were placed on the removed vials and they were labelled as to area, type of host (stump, windfall), trap position, and collection date.

Collected beetles were transported to the laboratory on ice and kept refrigerated (about 3–5°C) until processed. One beetle was randomly selected from each collection for microbial observations. For beetles collected from windfalls, the width of the prothorax was measured at its widest point using a calibrated ocular micrometer with an accuracy of 1/20 to 1/30 mm and the sex of the beetle was determined using the characteristic shape of the 7th abdominal tergite (Lyon 1958). Body colour (black or two-toned) was also recorded for beetles collected from windfalls in 1977 through 1979. Pronotal width and sex were also determined for beetles collected from stumps in area A in 1975.

Microbial isolations from beetles were made in a sterile laminar flow hood, on the surface of 0.2% Czapek's – Dox (Difco) in 2.0% Bacto Agar in 6-cm-diameter disposable plastic Petri plates. Plates were poured to a depth of 2 to 3 mm to facilitate light transmission for microscopy. Following gelation, agar plates were inverted and kept 7 to 10 days in a closed container with excess paper towels to minimize condensation and produce a 'droplet-free' agar surface free of excess liquid water. Prior to culturing, beetles were examined with a stereo microscope at 10X to 40X magnification for the presence of phoretic mites, which were enumerated and removed. In 1974, beetles were walked and tumbled on the agar surface; all subsequent cultures were of dissected beetles. The head, legs, elytra, thorax, and abdomen were dispersed on the agar after the beetle's tarsi had been touched to the surface. The anterior ventral surfaces of detached elytra were examined for cocoons of nematodes, which were enumerated and removed. Plates were incubated inverted in darkness at ambient room temperature and examined after 3, 7, and 14 days. Bacteria and fungi were examined with bright-field, dark-field, and phase microscopy directly on the agar surface in water under a 1-cm-square cover glass. For oil immersion observations, samples of cultures were mounted in water on a microslide, and a cover glass was applied and fixed to the slide with nail polish. The most common filamentous fungus was identified according to Crane and Schoknecht (1973). Other filamentous fungi (OFF) that were likely not close associates of the spruce beetle were identified to genus or family according to Barnett and Hunter (1972), Davidson (1954), and Griffin (1968). The presence of bacteria and yeasts was confirmed by examining samples in micro-filtered sterile water on a clean microslide at 940X to 1220X magnification using dark-field and phase illumination microscopy (Poindexter 1971). Bacteria were classified as *Actinomycetes* or non-*Actinomycetes* bacteria.

Data were pooled by year. The effect of area (A vs. B), host type (stump vs. windfall), sex, and beetle colour (black or two-toned) on pronotal width (beetle size) were tested using Student's t-tests (data were pooled across variables not being tested). The frequencies of the dominant filamentous fungus (*Pesotum* sp. A, see Results), other filamentous fungi (OFF), yeasts and bacteria, and mites and nematodes were analyzed using Pearson's Chi-square by area, host type, beetle sex, and colour. Analyses were conducted using JMP™ 8.0 (SAS Institute, Cary, NC, USA) at a significance level of $P \leq 0.05$.

Simpson's diversity index (Simpson 1949) was used to indicate fungal diversity. The index C is defined as:

$$C = 1 - \sum_{i=1}^{i=S} P_i^2$$

where P_i is the relative abundance of species i and S is species richness, defined as the number of competing species in the community. Species were considered dominant if $P_i > 1/S$ (Camargo 1992). Diversity increases as C increases from 0 to 1.

Results

Data collected for individual beetles by year, area, and host type (stump/windfall) are listed in Appendix A Tables A1 and A2. A total of 221 beetles were examined for microorganisms: 145 beetles were sampled from area A and 75 beetles were sampled from area B. Due to human error, the area could not be determined for one beetle (listed as area "A&B" in Appendix). Overall, 61 beetles were sampled from stumps and 160 beetles were sampled from windfalls. These beetles included 96 females and 71 males. Sixty-one beetles were two-toned in colour and 22 were all black. Because pronotal widths, beetle colour and sex were not all recorded for every beetle each year, sample size (n) is reported with statistics where it differs from those stated above.

Mean (n, SD) pronotal width of beetles in areas A and B was 2.31 mm (101, 0.14) and 2.33 mm (50, 0.11), respectively, and the difference was not significant ($t_{120} = 0.61$, $P = 0.54$). Mean (n, SD) pronotal width of beetles emerging from stumps and windfalls was 2.31 mm (7, 0.11) and 2.32 mm (145, 0.13), respectively, and this difference was not significant ($t_{149} = -1.42$, $P = 0.16$). Mean (n, SD) pronotal width of female and male beetles was 2.33 mm (89, 0.13) and 2.30 mm (62, 0.13), respectively, and this difference was not significant ($t_{149} = -1.42$, $P = 0.16$). Similarly, mean (n, SD) pronotal width did not vary with beetle colour ($t_{68} = -1.72$, $P = 0.09$). Mean (n, SD) pronotal width was 2.35 mm (52, 0.13) for two-toned beetles and 2.28 mm (18, 0.20) for all black beetles.

Pesotum sp. A was the most common filamentous fungus and was isolated from 63% of the 221 beetles sampled (Table 1). There was no significant difference in the incidence of *Pesotum* sp. A from beetles by area (A, 62%; B, 65%) ($\chi^2_1 = 0.23$, $P = 0.63$). *Pesotum* sp. A was isolated from 72% of beetles emerging from stumps and 59% of beetles emerging from windfalls, but the difference was not statistically significant ($\chi^2_1 = 3.08$, $P = 0.08$). *Pesotum* sp. A was isolated from 53% of females and 65% of males but this difference was not significant ($\chi^2_1 = 2.28$, $P = 0.13$). Similarly, the isolation frequency of *Pesotum* sp. A did not vary signficantly with colour morphotype; the fungus was isolated from 54% of two-toned beetles and 73% of all black beetles ($\chi^2_1 = 2.32$, $P = 0.13$).

A total of 305 isolations of other filamentous fungi (OFF) and *Actinomycetes*, representing at least 25 different species, were made from 221 beetles (Table 1). The incidence of OFF and *Actinomycetes* did not vary significantly by area, with 77% of beetles from area A and 79% of beetles from area B carrying at least one taxon ($\chi^2_1 = 0.13$, $P = 0.72$). However, incidence of OFF and *Actinomycetes* varied signficantly with host type, with 83% of beetles emerging from windfalls and 64% of beetles emerging from stumps carrying at least one species ($\chi^2_1 = 8.70$, $P = 0.003$). OFF and *Actinomycetes* averaged 1.2 and 1.5 taxa per beetle from stumps and windfalls, respectively. Three taxa were unique to beetles from stumps, while 11 taxa were unique to beetles from windfalls (Table 1). After *Pesotum* sp. A, *Penicillium* was the most common taxon and was isolated from 50% of all beetles, followed by unknown mycelioid species (20%), *Cladosporium* (18%), *Mucor* (10%), *Actinomycetes* (7%), and an unknown *Batrachospermum*-like species (5%) (Table 1). In addition to *Pesotum* sp. A, these OFF were

the dominant filamentous fungi isolated from beetles in stumps or windfalls (Table 1). The remaining OFF were each isolated from fewer than 5% of the beetles and many taxa were isolated only from a few beetles. The assemblages of filamentous fungi had high diversity in both host types as indicated by Simpson's diversity index (Table 1).

Yeasts and non-*Actinomycete* bacteria were isolated from 97% of beetles emerging from stumps; neither microorganism was isolated from the other 3% of beetles. Yeasts and non-*Actinomycete* bacteria were isolated from 78% of beetles emerging from windfalls, while only yeasts were isolated from 8% of these beetles, only bacteria from 9%, and neither microorganism from the remaining 5%. There was no significant difference between stumps and windfalls in the percentage of beetles without either microorganism ($\chi^2_1 = 0.51$, $P = 0.47$).

Both mites and nematodes were observed on the exoskeletons of 3% of beetles emerging from stumps, while only mites were observed on 2% of these beetles, only nematodes on 10%, and neither organism on 85%. Both mites and nematodes were observed on the exoskeleton of only one beetle (< 1%) emerging from windfall, while only mites were observed on 13% of beetles from windfall, only nematodes on 6% of windfall beetles, and neither organism was observed on 81% of windfall beetles. There was no significant difference between stumps and windfalls in the percentage of beetles without either organism ($\chi^2_1 = 0.48$, $P = 0.49$).

Discussion

Yeasts and bacteria were the most common associates of the spruce beetle as both types of microorganisms were isolated from the majority of beetles, regardless of host type. Isolations likely included a number of taxa, so it is not possible to determine the most common associate on an individual species basis. Previous studies have also reported that yeasts, as a group, were the most common associate of the spruce beetle (Ohsawa et al. 2000; Six and Bentz 2003; Aukema et al. 2005). Yeasts and bacteria may interact with other microbial associates of bark beetles, detoxify host tree compounds, contribute to pheromone synthesis, or alter the nutritional quality of tree tissues (e.g., Callaham and Shifrine 1960; Whitney 1971; Bridges 1981; Safranyik et al. 1983; Hunt and Borden 1990; Adams and Six 2008). Recent research has demonstrated the anti-fungal properties of *Actinomycetes* bacteria associated with the spruce beetle and the southern pine beetle (*Dendroctonus frontalis* Zimmerman) (Cardoza et al. 2006a; Scott et al. 2008). These microbes may inhibit certain antagonistic, gallery-invading fungi, or prevent the overgrowth of ophiostomatoid fungi (Cardoza et al. 2006a; Scott et al. 2008).

Pesotum sp. A, a blue-staining species, was the most common filamentous fungus associated with the spruce beetle. This fungus matched the morphological description of *Pesotum piceae* J.L. Crane & Schokn. (Crane and Schoknecht 1973), the anamorph of *Ophiostoma piceae* (Munch) Syd. & P. Syd. *Ophiostoma piceae* is one of nine currently recognized, closely related species in the *O. piceae* complex (Harrington et al. 2001). It is a globally distributed, ubiquitous, weakly pathogenic sapstain on conifers that may be dispersed by a wide variety of insects (Uzunovic et al. 2000; Harrington et al. 2001). *Ophiostoma piceae* has been isolated from many species of bark beetles, including the spruce beetle, although its frequency of association varies widely among and within bark beetle species (e.g., Haberkern et al. 2002; Six and Bentz 2003; Kirisits 2004; Aukema et al. 2005; Romón et al. 2007). Some fungi in the *O. piceae* complex in older studies may have been misidentified relative to current taxonomy due to the controversy surrounding the *O. piceae* complex, the presence of cryptic sibling species and changes in species delimitations that have occurred over time (e.g., Seifert 1993; Brasier and Kirk 1993; Uzunovic et al. 2000; Harrington et al. 2001). Thus, in lieu of phylogenetic and taxonomic analysis, a conservative approach is followed here and *Pesotum* sp. A is retained for the fungus isolated in this study. Regardless of species designations, fungi in the *O. piceae* complex are closely related and those inhabiting conifers appear to be common, non-species-specific associates of bark beetles.

Other studies reported that *Leptographium abietinum* (Peck) Wingfield was the most common filamentous fungus associated with the spruce beetle (Davidson 1954, 1955;

Hinds and Buffam 1971; Solheim 1995; Ohsawa et al. 2000; Six and Bentz 2003; Aukema et al. 2005). However, Rumbold (1936) found that *O. piceaperdum* (Rumb.) Arx was the most common associate. Other ophiostomatoid associates include *O. truncicolor* Davidson, *O. olivaceum* Mathieson, *O. piliferum* (Fries) H.P. Sydow, and the virulent pathogen *Ceratocystis rufipenni* Wingfield, Harrington & Solheim (= *C. coerulescens* [Münch] Bakshi in Davidson 1954, 1955), but *C. rufipenni* has only been isolated from tree tissues (Davidson 1954, 1955; Hinds and Buffam 1971; Safranyik et al. 1983; Solheim and Safranyik 1997; Wingfield et al. 1997). Aukema et al. (2005) also isolated multiple *Pesotum* anamorphs that were not identified to species. Interestingly, the three most prevalent associates identified to date, *O. piceae*, *L. abietinum*, and *O. piceaperdum*, are all found in association with many different species of bark beetles. These fungi may potentially provide similar or interchangeable benefits to bark beetles, e.g., colonizing vascular tissues and weakening tree defences. Although not considered highly pathogenic, the fungi elicit a wound response in trees that results in necrotic phloem and resin-soaked sapwood shortly after their inoculation (e.g., Safranyik et al. 1983; Lieutier 1993; Ross and Solheim 1997; Solheim and Safranyik 1997).

Cardoza et al. (2008) indicate that the interaction between *L. abietinum* and the spruce beetle may range from mutualistic to antagonistic and be context-dependent. Environmental or host variation over the range of the spruce beetle may affect the nature of the interaction and select for one species of fungus over another, resulting in spatial and temporal differences in the fungi associated with beetle populations (Hofstetter et al. 2007; Six and Bentz 2007). The spruce beetle lacks pocket- or sac-type mycangia found in some other bark beetles such as the mountain pine beetle (Whitney and Farris 1970). Fungal spores are carried phoretically on the exoskeleton of spruce beetles or under the elytra where they may be associated with nematodes (Cardoza et al. 2006b). The mode of transport and the generalist nature of the fungal associates may enable changes in the fungal flora in response to geographic or environmental factors. Although the fungal species may vary, spruce beetle populations sampled to date have had a high frequency of association with a blue staining, ophiostomatoid fungus, indicative of a beneficial relationship.

Numerous other filamentous fungi were isolated from the spruce beetle, although most were isolated at low frequencies (Table 1). The genera *Graphium*, *Leptographium*, *Sporothrix*, *Phialophora*, *Verticillium*, and *Phialocephala* are (or once were) genera containing ophiostomatoid fungi (Upadhyay 1993). These fungi may not be close associates of the spruce beetle because they were rarely isolated from beetles. However, in both the southern pine and mountain pine beetle (*Dendroctonus ponderosae* Hopkins) systems, which each include more than one fungal associate, the relative abundances of the different species varied to some extent geographically and temporally (Hofstetter et al. 2007; Six and Bentz 2007). A few taxa, including *Mucor*, *Cladosporium* and *Penicillium* spp., were frequently isolated from spruce beetles. Some species in these genera are ubiquitous, wind-dispersed fungi that are harmful to adults and developing brood; their rapid growth in culture also inhibits the growth of ophiostomatoid fungi (Jassim et al. 1990; Cardoza et al. 2006a; Bleiker and Six 2007). Thus, these species are not mutualists of bark beetles and are likely only incidental associates. The "unknown" group of filamentous fungi contained a number of asporogenous forms; some of these were likely new taxa and may have included some *Basidiomycete* species, while others may have been non-sporulating strains of several of the 25 taxa listed in Table 1.

Relatively few spruce beetles were associated with mites and/or nematodes, but both of these organisms were commonly observed in beetle galleries. Only mites and nematodes observed on the exteriors of beetles were recorded and individuals in cryptic locations would have been missed. Cardoza et al. (2008) found that nematodes were associated with 75% of the spruce beetles sampled, but that they were most often found under the elytra or within nematangia on the membranous hindwings. The origin of nematangia has yet to be determined, but Cardoza et al. (2006b) postulate that the leathery structures associated with, but not permanently affixed to, the hindwings are overwintering domatia for female nematodes, produced by interactions between substances produced by the beetles, fungi and nematodes. Mites and nematodes are common associates of bark beetles; species may be parasitic, predatory or mycophagous (e.g., Massey 1974; Moser 1975; Moser et al. 2005). Mites and nematodes may also be associated with microbes, including ophiostomatoid fungi, which may interact with bark beetles and their associated microorganisms, resulting in complex relationships that may affect bark beetle fitness and population dynamics (e.g., Moser 1985; Bridges and Moser 1986; Hofstetter et al. 2006a, b; Cardoza et al. 2008).

Based on Simpson's index, the diversity of microorganisms associated with the spruce beetle did not vary between host types (Table 1). Although more taxa were unique to beetles emerging from windfalls compared to stumps (11 versus 3 taxa, respectively), and there were more unknown species isolated from beetles emerging from windfalls, this may have been a product of the larger sample size from windfalls. Similar microbial diversity between host types may be due to either a consistent association between the insect and certain microbes (e.g., *Pesotum* sp. A) or the fact that some of the species are ubiquitous opportunists (e.g., *Penicillium* spp.).

Differences in tree tissues in stumps and windfalls may explain why the isolation frequency of *Pesotum* sp. A did not vary with host type, but the incidence of OFF was higher for beetles from windfalls. The phloem and sapwood likely remained moister in windfalls than in stumps because windfalls typically fell into stands where they were shaded by standing trees, which would have slowed their drying, while stumps were exposed in clearcuts. In addition, some of the roots of windfalls remained partially intact, delaying tree death. Some windfalls actually accrued some annual growth and displayed host resistance by producing pitch tubes in response to bark beetle attack. In contrast, stumps exposed in clearcuts dried rapidly and did not grow or produce pitch tubes. Tree tissues are first colonized by ophiostomatoid fungi, which are adapted to conditions in living and recently killed trees. Other fungi colonize tree tissues after the ophiostomatoid fungi. Moister conditions in windfalls compared to stumps at the time of beetle emergence likely favoured the growth of non-ophiostomatoid fungi in these hosts prior to beetle emergence. Low moisture conditions may have limited the growth of these fungi in stumps, while permitting the survival of ophiostomatoid fungi, which are tolerant of low moisture conditions (as low as 20% [Seifert 1993]). In addition, certain fast-growing fungi may overrun ophiostomatoid fungi growing in culture and obscure their presence (Bleiker and Six 2007). Thus, the isolation frequency reported for *Pesotum* sp. A is likely conservative, especially for beetles emerging from windfalls. This would also explain why the isolation frequency of *Pesotum* sp. A was 13% higher for beetles from stumps, although the difference was not statistically significant ($P = 0.08$).

The average widths of the pronotum of the two colour morphotypes and of the two sexes were within the ranges reported previously (males, 2.42–2.33 mm; females, 2.37–2.26 mm) (Safranyik and Linton 1983). However, the difference in the average size of the two colour morphotypes was tending towards statistical significance ($P = 0.09$), which may be an expression of either genetic or nutritional differences (Linton et al. 1984). The frequency of *Pesotum* sp. A did not vary significantly with colour morphotype or sex, indicating that however this fungus is transported by the beetle it is not affected by either of these factors.

On an evolutionary time scale, logging waste and stumps are very recent developments in the environment of spruce beetle populations. These host materials are more exposed to the elements than are windfalls in stand interiors and generally have greater variation in temperature, wind, moisture, and light conditions. Although fresh spruce stumps are readily attacked by spruce beetles, they are also highly attractive to other bark beetle species. For example, stumps become attractive to some species of *Hylurgops* and *Hylastes* that tend to prefer fermenting sap, weakened or dead trees, and that attack near or below the duff (Wood 1982). The differences in physical environments of stumps and windfall and in the relative abundances of associated bark beetle species with their own microorganism complexes can have considerable effect on the establishment, development and survival of the spruce beetles and their associated microorganisms. These are likely reasons for some of the differences between stumps and windfalls in the relative abundance of OFF observed in this study.

However, despite the foregoing statements about differences between stumps and windfalls as spruce beetle habitat, the constancy of association of *Pesotum* sp. A with the spruce beetle and the similarities between the relative abundances of the other associated microorganisms in the two host materials is remarkable. These similarities may explain, at least in part, why over an 8-year period in the experimental area there were no differences in the generation survival of the spruce beetle in the two types of host material (Safranyik and Linton 1999). The results also support the view that bark beetles and their associated microorganisms should be viewed as an interacting complex of species that inhabits trees (Whitney 1982).

Table 1. The frequency and relative abundance (P_i) of filamentous fungal taxa and isolated from spruce beetles emerging from stumps (n = 61 beetles) and windfalls (n = 160 beetles).

Taxon	Stumps	P_i	Windfall	P_i	Total	P_i
Pesotum sp. A	44	0.383*	95	0.289*	139	0.313*
Penicillium spp.	18	0.157*	94	0.286*	112	0.252*
Unknown species producing mycelium	9	0.078*	36	0.109	45	0.101*
Cladosporium spp.	10	0.087*	29	0.088*	39	0.088*
Mucor spp.	6	0.052*	17	0.052*	23	0.052*
Actinomycetaceae spp.	2	0.017	13	0.040*	15	0.034
Batrachospermum-like spp.	9	0.078*	2	0.006	11	0.025
Phoma spp.	1	0.009	8	0.024	9	0.020
Cephalosporium (= *Acremonium*) spp.	2	0.017	6	0.018	8	0.018
Stachybotrys spp.	2	0.017	4	0.012	6	0.014
Graphium spp.	2	0.017	4	0.012	6	0.014
Epicoccum spp.	4	0.035	0	0.000	4	0.009
Scopularia (= *Leptographium*) spp.	4	0.035	0	0.000	4	0.009
Sporotrichium (= *Sporothrix*) spp.	0	0.000	3	0.009	3	0.007
Phycomycetaceae spp.	0	0.000	3	0.009	3	0.007
Trichoderma spp.	1	0.009	2	0.006	3	0.007
Botrytis spp.	0	0.000	2	0.006	2	0.005
Phialophora spp.	0	0.000	2	0.006	2	0.005
Alternaria spp.	0	0.000	2	0.006	2	0.005
Pullularia spp.	0	0.000	2	0.006	2	0.005
Goidanichiella sp.	0	0.000	1	0.003	1	0.002
Nodulisporium (= *Verticillium*) sp.	1	0.009	0	0.000	1	0.002
Aspergillus sp.	0	0.000	1	0.003	1	0.002
Geotrichum sp.	0	0.000	1	0.003	1	0.002
Phialocephala sp.	0	0.000	1	0.003	1	0.002
Sporobolomycetaceae sp.	0	0.000	1	0.003	1	0.002
All taxa except *Pesotum* sp. A		71		234		305
All taxa including *Pesotum* sp. A		115		329		444
Diversity index		0.803		0.809		0.814

*Dominant species. Species were considered dominant if $P_i \geq 1/S$, where P_i is the relative abundance of species i and S is the species richness, the number of competing species in the community (Camargo 1992).

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Appendix A

Table A1. Incidence of microorganisms associated with spruce beetles emerging from stumps by sample area, stump, location on stump, and year.

Area[1]	Tree	Location[2]	Beetles in trap[3]	Sex	Pronotal width (mm)	*Pesotum* sp. A	OFF and *Actinomycetes* isolated[4]	Yeasts and non-*Actinomycete* bacteria[5]	Nematodes and mites[6]	Date[7] y-m-d
A-CP2(1)	11	NB-1	1	-	-	yes	25	both	neither	74-6-10
	11	NB-1	1	-	-	yes	14,9,25	both	neither	
	4	NT-2	1	-	-	no	25	both	nematodes	
	4	NB-2	1	-	-	yes	8	both	neither	
	5	WT-2	1	-	-	yes	14,8	both	nematodes	
	5	-	1	-	-	no	none	neither	neither	
	5	-	1	-	-	yes	13,5	both	neither	
	5	NM-1	1	-	-	yes	5,14,8,9,25	both	neither	
B-CP1	18	SM-1	1	-	-	yes	8	both	nematodes	
	19	WB-1	1	-	-	yes	none	both	neither	
	19	-	1	-	-	yes	none	both	neither	
	21	ST-2	1	-	-	yes	none	both	neither	
A-CP2	12	EB-1	1	-	-	yes	none	both	neither	
	16	WM-1	1	-	-	yes	none	both	neither	
	30	EM-2	1	-	-	yes	5,13.	both	nematodes	
A-CP1	5	SB-2	1	-	-	yes	20	both	neither	74-6-16
	5	EB-1	1	-	-	no	2	both	neither	
	11	NM-1	1	-	-	yes	7,8,9,14	both	neither	
	11	NB-2	1	-	-	no	none	both	neither	
A-CP2	1	NT-2	1	-	-	yes	none	both	neither	
	9	SB-1	1	-	-	no	none	both	neither	
	11	SB-1	1	-	-	yes	none	both	neither	
	30	WB-2	1	-	-	yes	none	both	neither	
	32	WT-1	1	-	-	yes	none	both	neither	
B-CP2(2)	12	ET-1	1	-	-	yes	20,25	both	neither	
	13	WB-1	1	-	-	no	13	both	neither	
	15	WB-1	1	-	-	yes	none	both	neither	
	15	WM-1	1	-	-	yes	none	both	neither	
	15	ET-1	1	-	-	yes	20,24	both	neither	
	16	ET-1	1	-	-	yes	14	both	neither	
	16	WT-2	1	-	-	yes	14	both	neither	
	16	EB-2	1	-	-	no	20	both	neither	
	17	ET-2	1	-	-	no	12	both	neither	
	17	ET-1	1	-	-	yes	14	both	neither	
	18	NM-1	1	-	-	yes	2,14	both	neither	
	19	EM-1	1	-	-	yes	8,14	both	neither	
	21	SB-2	1	-	-	yes	none	both	neither	
A-CP2(1)	5	NM-1	1	-	-	yes	none	both	neither	74-6-17
A-CP2(1)	11	NM-1	1	-	-	yes	none	both	neither	74-6-17
	11	NM-2	1	-	-	yes	14,25	both	neither	
	32	EB-2	1	-	-	no	1	both	neither	
A-CP1	21	SB-1	1	-	-	yes	25	both	neither	
A-CP2(2)	12	WT-1	1	-	-	yes	none	both	neither	
A-CP2	2	NB-1	1	-	-	no	none	both	neither	74-7-8
	45	-	1	-	-	yes	none	both	neither	

Table A1. ... cont. Incidence of microorganisms associated with spruce beetles emerging from stumps by sample area, stump, location on stump, and year.

Area[1]	Tree	Location[2]	Beetles in trap[3]	Sex	Pronotal width (mm)	*Pesotum* sp. A	OFF and *Actinomycetes* isolated[4]	Yeasts and non-*Actinomycete* bacteria[5]	Nematodes and mites[6]	Date[7] y-m-d
A-CP2(1)	4	-	1	-	-	no	25	both	neither	
	11	-	1	-	-	yes	14	both	neither	
B-CP2(2)	31	-	1	-	-	yes	25	both	neither	
A-CP2(1)	8	NT-2	1	F	2.43	yes	7,14,23	both	neither	75-5-4
	8	-	4	M	-	no	5,8,14	both	both	
	9	-	1	F	2.22	no	5,14	both	neither	
	9	NT-2	1	F	2.11	yes	5	both	neither	
	19	ST-2	1	M	2.38	no	none	both	nematodes	
	19	SM-2	1	F	2.38	no	23,14	both	neither	
	19	-	11	F	-	yes	5	both	nematodes	
	19	-	1	M	2.27	yes	14,17	both	neither	
	21	-	1	M	2.38	no	none	neither	neither	
B (stump)	1	B	1	-	-	no	5,8,9,13	both	neither	79-6-10
(stump)	2	B	1	-	-	yes	8,13,14	both	neither	
(stump)	2	B	1	-	-	yes	5,8,13	both	mites	
(stump)	3	T	1	-	-	yes	12,14	both	both	

[1] Adjacent timber harvesting licenses, A= 940 ha and B= 1640 ha; CP(l)= cutting permit number for clear-felled area. No entry denotes same area as previous entry

[2] Location of paired 10-cm-diameter emergence traps. Traps were placed near the top (T), middle (M) and bottom (B) of stumps in the cardinal directions (N, E, S, W).

[3] Total number of beetles in emergence trap. One beetle was randomly selected from each emergence trap and examined for microorganisms.

[4] Taxa of OFF (filamentous fungi other than *Pesotum* sp. A) and *Actinomycetes* isolated from spruce beetles emerging from stumps or windfalls were coded as follows: 1, *Nodulisporium* (=Verticillium); 2, *Actinomycetaceae*; 3, *Alternaria*; 4, *Aspergillus*; 5, *Batrachospermum*-like; 6, *Botrytis*; 7, *Cephalosporium* (=*Acremonium*); 8, *Cladosporium*; 9, *Epicoccum*; 10, *Geotrichum*; 11, *Gonatobotrys*; 12, *Graphium*; 13, *Mucor*; 14, *Penicillium*; 15, *Phialocephala*; 16, *Phialophora*; 17, *Phoma*; 18, *Phycomycetaceae*; 19, *Pullularia*; 20, *Scopularia* (=*Leptographium*); 21, *Sporobolomycetaceae*; 22, *Sporotrichum* (=*Sporothrix*); 23, *Stachybotrys*; 24, *Trichoderma*; and 25, unknown mycelioid species.

[5] The presence of yeast(s) and bacteria other than *Actinomycetes* was recorded as follows: both, yeast and bacteria were present; bacteria, bacteria were isolated without yeast; yeast, yeast(s) was isolated without bacteria; or neither, neither yeasts nor bacteria were isolated.

[6] The presence of mites and nematodes on exoskeletons was recorded as follows: both, mites and nematodes were present; mites, only mites were observed; nematodes, only nematodes were observed; or neither, neither mites nor nematodes were observed.

[7] Date isolations made from beetles. No entry denotes same date as previous entry.

Table A2. Incidence of microorganisms associated with spruce beetles emerging from windfalls by sample area, tree, location on windfall, and year.

Area[1]	Tree	Location[2]	Beetles in trap[3]	Sex	Pronotal width (mm)	Colour	*Pesotum* sp. A	OFF and *Actinomycetes* isolated[4]	Yeasts and non-*Actinomycete* bacteria[5]	Nematodes and mites[6]	Date[7] y-m-d
A&B	-	-	32	M	2.31	-	no	2,14,15	both	nematodes	75-5-4
A	8	-	11	F	2.23	-	no	7, 8, 17, 25	both	nematodes	
	8	-	8	F	2.22	-	no	23,14	bacteria	neither	
	12	-	2	M	2.19	-	no	none	bacteria	neither	
	14	-	6	M	2.31	-	yes	17	both	neither	
	14	-	6	F	2.22	-	no	25	both	neither	
	14	-	2	F	2.30	-	no	8,14	both	neither	
	15	-	4	M	2.26	-	no	14,16,17,21	both	neither	
	19	-	14	M	2.29	-	yes	2,8,14	both	neither	
	19	-	6	M	2.24	-	yes	8,14	both	neither	
	20	-	1	M	2.16	-	no	6,13,14	both	neither	
	21	-	1	M	1.16	-	yes	5,14,22	both	neither	
B	3	-	3	M	-	-	no	17	both	neither	
	3	-	1	M	2.43	-	no	none	bacteria	neither	
	3	-	1	M	2.27	-	yes	none	both	nematodes	
	8	-	2	F	2.35	-	yes	2,8,13,17	both	neither	
	8	-	2	F	2.30	-	yes	7,14	both	neither	
	11	-	1	M	2.27	-	yes	7,14,17,22	both	neither	
	11	-	2	F	2.22	-	no	14,17	both	neither	
	11	-	1	M	2.22	-	no	13	bacteria	neither	
A	-	-	1	F	2.26	-	no	8,14	neither	neither	76-5-21
	-	-	9	F	2.22	-	yes	8,14	both	neither	
	5	-	1	M	2.31	-	yes	7,14,19	bacteria	neither	
	7	-	1	F	2.44	-	yes	8	both	neither	
	8	-	3	F	2.53	-	yes	2	both	neither	
	15	-	1	M	2.26	-	yes	2,8,14	both	neither	
B	1	1	1	M	2.44	-	yes	14,18,25	yeast	neither	76-5-31
	1	1	1	F	2.39	-	yes	25	both	neither	
	8	-	1	F	2.35	-	yes	2,14	both	neither	
	8	-	1	F	2.40	-	yes	4,14	both	neither	
	8	8	1	M	2.44	-	yes	14,25	both	neither	
	9	-	1	M	2.26	-	no	14,25	yeast	neither	
	9	8	2	F	2.35	-	yes	14	both	neither	
A	1	2	1	F	2.35	-	yes	14	both	mites	
	2	6	2	F	2.42	-	yes	none	both	neither	
	3	3	1	M	2.44	-	yes	none	both	neither	
	4	2	2	F	2.37	-	no	none	both	nematodes	
	4	3	1	M	2.40	-	yes	14	both	neither	
	5	5	2	M	2.35	-	no	14,18	both	neither	
	5	5	1	M	2.40	-	no	25	both	mites	
	6	4	1	F	2.44	-	yes	none	both	neither	
	14	-	1	F	2.04	-	yes	12,14	both	mites	
	15	-	1	F	2.26	-	yes	none	yeast	neither	
	3	1	1	F	-	-	yes	8,14	both	neither	76-6-5
	3	1	2	M	2.31	-	yes	none	both	mites	
	4	2	2	F	2.31	-	yes	23,10,14,25	both	neither	
	4	3	1	M	2.09	-	yes	none	both	neither	
	8	2	1	F	2.31	-	no	25	both	neither	
	10	1	1	F	2.40	-	yes	14	both	neither	
	10	2	1	F	2.13	-	no	13,14	both	neither	

Table A2.... cont. Incidence of microorganisms associated with spruce beetles emerging from windfalls by sample area, tree, location on windfall, and year.

Area[1]	Tree	Location[2]	Beetles in Trap[3]	Sex	Pronotal width (mm)	Colour.[4]	*Pesotum* sp. A	OFF and *Actinomycetes*[5]	Yeasts and non-*Actinomycete* bacteria[6]	Nematodes and mites[7]	Date[8] y-m-d
B	3	3	4	M	2.30	-	yes	11	both	neither	
	7	1	1	F	2.09	-	yes	14	both	neither	
	7	1	1	F	2.17	-	no	16	neither	neither	
	7	2	1	F	2.40	-	no	2,14	bacteria	neither	
	8	1	1	M	2.26	-	yes	2,7	both	mites	
	8	4	1	M	2.31	-	yes	14,23	both	mites	
	9	5	1	M	2.49	-	yes	8,14	both	mites	
	9	8	2	F	2.31	-	yes	none	both	neither	
A	4	2	4	M	2.27	-	no	14	both	mites	
	6	4	2	F	2.26	-	yes	none	both	mites	
	10	-	1	M	2.44	-	yes	8,13,14	both	neither	
	13	5	1	F	2.49	-	no	2,14	both	both	
B	9	4	1	F	2.26	-	no	14,24	both	neither	76-6-7
A	3	3	1	F	2.40	-	no	8,13,14,18,24	neither	neither	76-6-9
	4	2	1	F	2.44	-	no	7,14	both	neither	
	9	4	1	F	2.35	-	yes	2,8,14	bacteria	neither	
	10	1	1	M	2.53	-	yes	2,13,14	both	neither	
	13	1	1	F	2.40	-	no	8,13	both	mites	
B	3	2	1	F	2.35	-	yes	14	neither	nematodes	
	7	2	1	F	2.26	-	no	8,14,25	bacteria	neither	
A	4	2	1	F	2.40	-	no	2,25	both	neither	76-6-18
	7	2	1	M	2.22	-	yes	8,14,19	both	neither	
	10	1	2	F	2.40	-	no	13,14,25	both	neither	
	10	1	1	F	2.26	-	yes	14	both	neither	76-6-24
	10	2	1	M	2.26	-	yes	2,12,14,25	both	neither	76-7-9
	1	6	1	F	2.40	-	no	8,14,23	yeast	mites	76-7-14
	-	-	1	F	2.22	-	yes	8,14	both	neither	
	3	5	1	M	2.33	2t	no	14	bacteria	neither	77-5-29
	3	-	1	F	2.33	2t	yes	14	yeast	neithe	
B	2	-	1	F	2.33	blk	yes	14	both	neither	
	2	-	2	M	2.40	2t	yes	none	yeast	neither	
	2	-	1	F	2.40	2t	yes	8,14	both	neither	
	2	-	5	M	2.23	2t	yes	14	both	neither	
	4	3	3	M	2.36	2t	no	14	yeast	neither	
	5	4	1	F	2.46	2t	yes	none	both	neither	
	6	-	5	M	2.26	2t	yes	none	both	nematodes	
A	2	-	1	F	2.33	2t	yes	none	both	neither	
	3	5	1	M	2.26	2t	yes	8,14	both	neither	
	1	-	1	F	2.46	2t	no	3,14	both	neither	77-6-6
	1	-	3	F	2.46	2t	no	3,14	both	neither	
	1	-	3	F	2.40	2t	no	13,14	both	neither	
	1	4	1	F	2.50	blk	yes	14	both	neither	
	3	2	2	M	2.30	blk	no	14	neither	mites	
	3	5	1	F	2.13	2t	yes	6,14	both	neither	
	6	5	5	F	2.53	2t	no	12,14	yeast	mites	
	7	1	1	F	2.46	2t	no	14	neither	mites	
B	1	-	1	M	2.36	2t	no	14	yeast	neither	
	2	-	2	F	2.50	2t	yes	14	both	neither	
	3	6	2	M	2.30	blk	no	14	bacteria	neither	
	6	-	1	M	2.40	2t	yes	14,25	yeast	neither	

Table A2.... cont. Incidence of microorganisms associated with spruce beetles emerging from windfalls by sample area, tree, location on windfall, and year.

Area[1]	Tree	Location[2]	Beetles in Trap[3]	Sex	Pronotal width (mm)	Colour.[4]	*Pesotum* sp. A	OFF and *Actinomycetes*[5]	Yeasts and non-*Actinomycete* bacteria[6]	Nematodes and mites[7]	Date[8] y-m-d
A	2	-	2	F	2.38	2t	no	14,25	both	neither	77-6-14
	3	-	5	F	2.16	2t	yes	14	both	neither	
	4	-	1	F	2.33	2t	no	14,25	both	neither	
	1	-	1	F	2.46	2t	no	14	both	neither	77-6-26
	2	-	1	F	2.16	2t	no	8,12,14	both	neither	
	6	-	2	F	2.53	2t	no	14	both	neither	
	6	-	2	F	2.60	2t	no	14	neither	neither	
A	3	3,6	4	M	2.65	blk	yes	none	both	mites	78-5-30
	4	5	2	M	2.41	2t	yes	14,25	both	neither	
	4	2	1	F	2.35	2t	yes	25	both	nematodes	
	6	1	5	F	2.16	2t	yes	25	both	neither	
	8	2	2	F	2.59	2t	no	14	bacteria	neither	
	9	6	1	F	2.47	2t	no	13	both	neither	
	10	6	3	F	2.53	blk	yes	25	yeast	neither	
B	2	3	2	F	2.10	2t	no	none	neither	mites	
	3	2	1	F	2.16	2t	no	14	bacteria	neither	
	6	5	1	F	2.22	2t	no	14	neither	mites	
	7	2	1	M	2.27	2t	yes	14	both	neither	
	7	5	1	F	2.58	2t	no	14,25	both	neither	
	8	6	8	M	2.41	2t	yes	17	both	neither	
	10	1	1	F	2.47	2t	yes	none	both	nematodes	
	4	5	2	M	2.16	blk	no	14	yeast	neither	78-6-12
	4	-	1	F	2.22	2t	no	13,14	both	neither	
	10	-	1	M	2.53	2t	no	14,25	both	neither	
A	4B	3	2	M	2.35	2t	no	25	bacteria	neither	
	4	4,1	2	F	2.47	2t	yes	25	both	neither	
	10	5	1	M	1.85	blk	yes	25	both	neither	
	11	1,5	2	F	2.47	2t	yes	25	both	neither	
B	6	4	3	F	2.47	blk	no	25	both	neither	
	6	4	1	F	2.28	2t	no	22	both	neither	
	10	5	6	M	2.47	2t	yes	none	both	neither	
	10	6	1	M	2.35	2t	no	none	bacteria	mites	

Table A2.... cont. **Incidence of microorganisms associated with spruce beetles emerging from windfalls by sample area, tree, location on windfall, and year.**

Area[1]	Tree	Location[2]	Beetles in Trap[3]	Sex	Pronotal width (mm)	Colour.[4]	*Pesotum* sp. A	OFF and *Actinomycetes*[5]	Yeasts and non-*Actinomycete* bacteria[6]	Nematodes and mites[7]	Date[8] y-m-d
A	5	4	2	M	2.35	2t	yes	none	both	neither	79-6-5
	5	5	2	M	2.25	blk	yes	25	both	neither	
	5	6	1	M	2.06	blk	yes	25	both	neither	
	6	1	1	F	2.19	blk	yes	8,25	both	neither	
	6	1,2,3	3	F	2.40	blk	no	25	both	mites	
	6	2,3	3	-	2.41	blk	yes	8,25	both	neither	
	6	6,4	2	M	2.09	blk	yes	none	both	neither	
	7	3,4	3	F	2.07	2t	yes	8,25	both	neither	
	7	5,3,2	7	M	2.19	2t	no	none	both	neither	
	7	3	4	M	2.09	blk	yes	none	both	neither	
	9	2	2	F	2.19	2t	yes	none	both	neither	
	9	2	1	F	2.22	2t	yes	none	both	neither	
	12	1	1	F	2.19	blk	yes	14,25	both	mites	
	13	2,4	11	M	2.28	2t	yes	13,25	both	neither	
	13	2,4,6	4	F	2.28	blk	yes	none	both	neither	
	-	-	1	M	-	2t	yes	5	both	neither	
	-	-	1	M	-	2t	yes	8,14	both	neither	
	-	-	1	M	-	2t	yes	14	both	neither	
	-	-	1	F	-	blk	yes	13	both	neither	
	-	-	1	F	-	blk	no	13,14	both	neither	
	-	-	1	F	-	2t	no	13,14	both	neither	
	-	-	1	M	-	2t	yes	8,14	both	neither	
	-	-	1	F	-	blk	yes	14	both	neither	
	-	-	1	M	-	2t	no	13,14	yeast	neither	
	-	-	1	M	-	2t	yes	14	both	neither	
	-	-	1	M	-	blk	yes	8,14	both	neither	
B	-	-	1	-	-	2t	yes	25	both	neither	79-6-10
B	-	-	1	F	-	2t	yes	8	both	mites	79-6-10

[1] Adjacent timber harvesting areas, A = 940 ha and B = 1640 ha. No entry denotes same area as previous entry.

[2] Location of paired 10-cm-diameter emergence traps. Traps were placed along both sides of windfalls near the mid-point of each quarter section of the beetle-infested stem starting with locations 1 and 2 nearest the base of the tree.

[3] Total number of beetles in emergence trap. One beetle was randomly selected from each emergence trap and examined for microorganisms.

[4] Colour of beetles was either blk (all black) or 2t (two-toned, where the head and prothorax were black and the elytra were brown or reddish-brown).

[5] Taxa of OFF (filamentous fungi other than *Pesotum* sp. A) and *Actinomycetes* isolated from spruce beetles emerging from stumps or windfalls were coded as follows: 1, *Nodulisporium* (=*Verticillium*); 2, *Actinomycetaceae*; 3, *Alternaria*; 4, *Aspergillus*; 5, *Batrachospermum*-like; 6, *Botrytis*; 7, *Cephalosporium* (=*Acremonium*); 8, *Cladosporium*; 9, *Epicoccum*; 10, *Geotrichum*; 11, *Graphium*; 12, *Gliomastix*; 13, *Mucor*; 14, *Penicillium*; 15, *Phialocephala*; 16, *Phialophora*; 17, *Phoma*; 18. *Phycomycetaceae*; 19, *Pullularia*; 20, *Scopularia* (=*Leptographium*); 21, *Sporobolomycetaceae*; 22, *Sporotrichium* (=*Sporothrix*); 23, *Stachybotrys*; 24, *Trichoderma*; and 25, unknown mycelioid species.

[6] The presence of yeast(s) and bacteria other than was recorded as follows: both, yeast and bacteria were present; bacteria, bacteria were isolated without yeast; or yeast, yeast(s) was isolated without bacteria; or neither, neither yeasts nor bacteria were isolated.

[7] The presence of mites and nematodes on exoskeletons was recorded as follows: both, mites and nematodes were present; mites, only mites were observed; nematodes, only nematodes were observed; neither, neither mites nor nematodes were observed.

[8] Date isolations made from beetles. No entry denotes same date as previous entry.

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